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THE EFFICIENCY OF LEGUME INOCULATION FOR ARIZONA SOILS

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*THE EFFICIENCY OF LEGUME INOCULATION FOR ARIZONA SOILS

By R. S. HAWKINS, Assistant Agronomist

INTRODUCTION

A few years ago several companies handling commercial cultures of *Bacillus radicicola* began a more or less extensive advertising campaign for the sale of their products. At that time no data had been obtained as to the actual need of Arizona soils for artificial inoculation. In order to secure this data the Agronomy Department of the University of Arizona, College of Agriculture, outlined experiments in inoculation work which were carried on at the various Experiment Station farms in the State. In addition to the field work, detailed pot experiments were conducted at the University campus. The effect of inoculation was studied principally with regard to: (1) Yield; (2) increase in root growth, with its extra manurial value; (3) increase in nitrogen content of tops, roots, and of the soil in which the plants were grown.

The soils of the arid Southwest are very different from those of other sections of the United States and as yet little is known as to bacterial action in these soils. No attempt has been made in the present study to investigate the habits of the bacteria themselves further than an effort to determine the effect of the symbiotic nitrogen-fixing *Bacillus radicicola* upon the growth and nitrogen content of the various legumes studied. The methods used and the results obtained are recorded in this publication.

FIELD TESTS

SALT RIVER VALLEY EXPERIMENT FARM

The soil upon this farm is a tight-textured clay loam soil. In the fall of 1919 ten one-acre plots were planted to hairy Peruvian alfalfa, some of the plots being planted with inoculated seed and others with seed that had received no treatment. Soil from an old alfalfa field was scattered on one of the acre plots. At no time during the year could a noticeable difference in growth be observed. The yields for the years 1920 and 1921 show that the four acres planted with untreated seed averaged 132 pounds more to the acre per cutting than did the plots which had been planted with inoculated seed.

*Presented as a thesis in partial fulfillment for the work of a master's degree.

TABLE I. INOCULATION STUDIES ON A TEN-ACRE FIELD OF ALFALFA ON THE SALT RIVER VALLEY EXPERIMENT FARM NEAR MESA, ARIZONA.

Border Treatment No.		Yield cured hay per acre per average cutting		
		1920	1921	Average for 2 years
W57	none	1475	2056	1775
W58	none	1799	2196	1997
W59	Westrobac inoculation	1653	1760	1706
W60	Westrobac inoculation	1725	2607	2166
W61	Farmogerm inoculation	1903	2292	2097
W62	Soil transfer	1946	2385	2165
W63	Mulford inoculation	1729	2262	1995
W64	Mulford inoculation	1868	2271	2069
W65	none	2335	2850	2592
W66	none	2348	2271	2259
		Lbs.	Lbs.	Lbs.
Average of the four acres planted with untreated seed		1987	2343	2165
Average of the six acres planted with inoculated seed (Including soil transfer)		1804	2263	2033

Examinations of the roots of the alfalfa plants at several times during the season showed that nodules were present in approximately equal numbers, regardless of whether the seed had been artificially inoculated or not. Previous to the present planting of alfalfa, no legume crops had been grown on this land since the time that the University of Arizona purchased it in 1915.

TABLE II. INOCULATION STUDIES WITH COWPEAS AT THE SALT RIVER VALLEY EXPERIMENT FARM NEAR MESA, ARIZONA.

Treatment	Yield per acre, threshed seed		
	1920	1921	Average for 2 years
Farmogerm inoculation	222	105	163.5
Mulford culture inoculation	225		
Westrobac inoculation	294		
Not inoculated	165	76	120.0

There was a rank growth of stems in every case, both on the areas sown with inoculated seed and on the areas sown with untreated seed. Seed did not set in sufficient quantities on any of the borders to pay for the threshing. Red Ripper cowpeas were used for these tests.

A careful examination of the roots indicates that during the hot months of the year when cowpeas are ordinarily grown for green manuring purposes few or no nodules are developed, regardless of whether or not artificial inoculation has been used.

This same observation has been made with soybeans; namely, that nodules common to the roots of all legumes and necessary to these plants in order for them to utilize the atmospheric nitrogen through the agency of the *Bacillus radicicola,* are not present in quantity during the middle of the summer in the hot climate of the Salt River Valley.

SULPHUR SPRING VALLEY DRY-FARM

During the early part of the growing season of 1919 there was no noticeable difference between the inoculated plants and those not inoculated, in the tests conducted at the Sulphur Spring Valley Dry-Farm. Nodules were easily found on the roots of all the varieties. About fifteen days before harvest several of the inoculated plots showed gains over those not inoculated, and at harvest time they had gained 15.7 percent.

The season of 1920 was a very dry one, and all yields were so poor that comparative data were unsatisfactory. The inoculated plots made a gain of but 3.4 percent over the plots not inoculated.

Grasshoppers and rabbits injured the legume plots to such an extent during the season of 1921 that the results are not comparable.

From the data obtained and from the appearance of the plots before they were damaged by drought, insects, and rabbits during the last two seasons, indications are very strong that artificial inoculation is needed for maximum yields on this farm. The soil is a very poor clay soil with considerable sand in it. All of the legumes were grown each year on plots where no legumes had grown in previous years.

TABLE III INOCULATION TESTS AT SULPHUR SPRING VALLEY DRY FARM.

		Yields per acre		
		1919	1920	1921
		Forage	Seed	Seed
Early Bird Velvet beans	inoc.	1375		
	not inoc.	1320		
Chinese Velvet beans	inoc.	1375		
	not inoc.	1100		
Black Eye cowpeas	inoc.	2090	88	
	not inoc.	1760	82	
Red Ripper cowpeas	inoc.	1155		451
	not inoc.	1265		407
New Era cowpeas	inoc.	2695		
	not inoc.	2475		
Mammoth Yellow soybeans	inoc.	770		
	not inoc.	770		
Pink beans	inoc.		104.5	220
	not inoc.		99	352
Red Ripper cowpeas	inoc.		71.5	
	not inoc.		71.5	
Taylor cowpeas	inoc.		38.5	
	not inoc.		44	
Groit cowpeas	inoc.			385
	not inoc.			429
Pinto beans	inoc.			102
	not inoc.			187
Tepary beans	inoc.			407
	not inoc.			517
Average gain or loss of inoculated plots		15.7%	3.4%	17.7%

YUMA MESA FARM

In the spring of 1921 cultivation was first begun on a part of the Experiment Station Farm located on the Yuma mesa. Vetch was used as a cover and green manure crop in the young citrus orchard during the first season. Prior to planting most of the seed was inoculated with a commercial culture, but some of the seed had received no treatment. Plants from inoculated

Fig. 1.—Vetch in the citrus orchard of the Yuma Mesa Farm. Borders on left planted with inoculated seed; on right planted with untreated seed. Photograph taken April 6, 1921.

seed made a growth several times greater than did plants from seed that was not inoculated. Not only was the growth heavier, but as the season progressed many of the plants not artificially inoculated died. An examination of the roots showed that the artificially inoculated plants had developed a much heavier root system and a greater abundance of nodules than had the plants which had received no such inoculation. Practically all of the plants which survived on the area sown with untreated seed had some root nodules present, which indicates that the *Bacillus radicicola* is present to some extent in raw desert soil, but apparently in quantities not sufficient to make possible a maximum inoculation of legumes, and hence for this result artificial inoculation must be practiced.

A mixture of vetch and rye was planted in the same orchard in the spring of 1922. A part of the area was planted with inoculated vetch seed, and the remainder with vetch seed

Fig. 2.—Vetch and rye on the Yuma Mesa Farm. First crop on land. Border on left planted with inoculated seed; on right planted with untreated seed. Photograph taken May 6, 1922.

not inoculated. At the present time, May 1, 1922, the growth of vetch is appreciably thicker on the area sown with inoculated seed than on the area where the seed was not inoculated, notwithstanding the fact that on both areas a heavy crop of inoculated plants had been plowed under the previous year. The soil in this orchard is uniformly sandy and contains little organic matter. One year's growth of inoculated legumes did not put the soil in condition to grow a maximum crop of vetch the next year without additional inoculation.

TABLE IV. THE EFFECT OF INOCULATION ON THE DRY WEIGHT OF THE TOPS, ROOTS, AND NODULES OF COMMON VETCH GROWN AT THE YUMA MESA FARM. AVERAGE OF FIVE REPRESENTATIVE PLANTS FROM EACH AREA. PLANTS COLLECTED MAY 6, 1922.

Soil and treatment	Dry weight in grams		
	Tops	Roots	Nodules
Cropped last year with artificially inoculated vetch.			
Inoculated this year	4.744	2.92	.173
Not inoculated this year	7.162	1.451	.286
Cropped for first time this year.			
Inoculated	4.228	1.492	.138
Not inoculated	2.283	1.01	.216

An examination of Table 4 shows that, although the artificially inoculated plants made a much heavier root growth than those not so inoculated, the weight of the nodules on the roots of the plants not artificially inoculated was on an average greater than the weight of the nodules on the roots of the plants that had been inoculated artificially. It was also noticeable that the nodules for the most part were larger on the plants depending on natural inoculation than on those artificially inoculated. On the area cropped for the first time this year, most of the plants which were not artificially inoculated died after a few weeks. The ones which did survive had an abundance of nodules.

Data taken from these surviving plants are not strictly comparable with data obtained from the plants grown from inoculated seed, since only the best plants survived in the first instance, while average plants were used in every other case.

A marked difference in appearance can be seen between the citrus trees on the area where a good crop of green manure grown from inoculated vetch seed was plowed under last year and those on the area where green manure grown from vetch seed that had not been inoculated had been plowed under. The vetch plants from the untreated seed made little growth for green manure. The leaves of the citrus trees on the former area were dark green in color, while those of the citrus trees of the latter area were light green in color.

POT EXPERIMENTS

Pot experiments were conducted at the University campus during the growing seasons of 1921 and 1922, to determine the possibility of increasing by inoculation the yields of legumes in certain soils found in the vicinity of Tucson; and also to determine the effect of inoculation on the nitrogen content of the tops and roots of the plants and of the soil in which the plants grew. If the plants had been grown in the field it would have been exceedingly difficult to obtain fair samples of the soils for analyses.

METHODS

Three different soils were used: (1) A sandy loam virgin soil from the Rillito River bottom; (2) a similar soil that has been under cultivation with a good rotation system for a number of years; and, (3) a sandy desert soil which has never been under cultivation. These soils were all carefully screened through a one-millimeter screen and thirteen kilograms of soil were put into each pot; all the pots had been coated previously on the inside with paraffin.

Two trenches, each about a foot deep, were dug in the screen garden where the work was to be done. In the bottom of each trench a platform, having twelve holes to hold the pots, was placed in such a manner that the bottoms of the pots

were prevented from coming in contact with the surrounding soil. Had the bottoms of the pots been placed in contact with the ground, bacteria, if present in the soil, could easily have entered through the drainage holes in the bottoms of the pots. Since the pots used were not glazed, the paraffin coating served to prevent the entrance of bacteria through the pores of the walls. After the pots containing the soils were in place on the platforms, dirt was carefully packed around them so as to fill completely the trenches above the platforms, leaving the pots protruding two or three inches above the surface of the ground. One set of twelve pots which was to be planted with inoculated seed was placed in a different section of the screen garden, approximately fifty feet from the other set which was to be planted with seed not inoculated. This was done to avoid, as far as possible, accidental inoculation.

Inoculation material was obtained from the United States Department of Agriculture, and the seed to be planted was given uniform treatment. The seed was sown on April 12, 1921, as follows:

Hairy Peruvian Alfalfa:

Virgin soil, Rillito River bottom	4 pots
Virgin soil, Rillito River bottom, sterilized*	2 pots
Cultivated Rillito River bottom soil	4 pots
Virgin desert soil	4 pots

Whippoorwill Cowpeas:

Virgin soil, Rillito River bottom	6 pots

Ito San Soybeans:

Virgin soil, Rillito River bottom	4 pots

One-half of the above pots was planted with inoculated seed and the other half with seed which had not been inoculated.

RESULTS

Notwithstanding that care commensurate with the equipment available was used to prevent accidental inoculation, some outside inoculation did occur, as is evidenced by the fact that the alfalfa plants growing in sterilized soil* from seeds which had not been inoculated, developed a few nodules on their roots. These pots of sterilized soil were used to ascertain the possible occurrence of accidental contamination, as well as for a check on the vitality of the inoculation material used. In the case of the soybeans which had not been inoculated, no root nodules could be found. In all other instances some root nodules were found on both the inoculated plants and on those not inoculated. The nodules were much more abundant, however, on the plants grown from inoculated seed than on those grown from seed that was not inoculated.

*Sterilized in autoclave for one hour at 20 pounds pressure.

The inoculated plants reached maturity in every instance five to seven days before those that were not inoculated. All the plants of each variety were harvested at the same time and the dry weights taken. In the case of the alfalfa, three cuttings during the season were made from the pots containing the inoculated plants, and only two cuttings from the pots containing the plants which had not been inoculated, since growth after the second cutting was not sufficient to warrant harvesting. After the last cuttings were made, samples of the soils were taken and the roots were carefully washed on a screen to free them from soil particles. The same procedure was used with the soybeans and cowpeas, the cuttings being made just before the formation of the seed, with the exception of two pots of cowpeas, which were not harvested until the seed had ripened.

In every instance there was a decided gain in weight of dry matter in the tops of the inoculated plants over those not inoculated. In the case of the alfalfa this difference was greatest in the plants grown in desert soil, the gain being almost 65 percent. The next greatest gain was with the sterilized virgin river bottom soil, the gain here being 59 percent. The same soil not sterilized before planting produced plants which made a gain in weight of 55 percent, due to inoculation, while the gain in weight of the plants grown in the cultivated soil was 58 percent. This indicates that, while inoculation was of value in each case, it was of greatest value on the poorer desert soil, at least as far as gain in dry matter was concerned.

The increase in dry matter due to inoculation was more pronounced in the first cuttings in each instance, with the exception of the plants grown in the sterilized soil, where the increase was greater by 20 percent in the second cutting. This indicates that bacterial growth and action from accidental contamination was increasing in the pots which had not received inoculation. This increase was progressing at a rate that would soon render the soil in these pots more nearly like that which had been given the benefit of the inoculation. The *Bacillus radicicola* must have been present in the original soils, and when the alfalfa seed was planted and water applied the bacteria soon multiplied, and by the time the alfalfa was cut the second time they had made a large increase over the number present when the first cuttings were made. This conclusion is further substantiated by the fact that in the case of the sterilized soil, the increase due to inoculation at the first cutting was 31 percent, and at the second cutting 51 percent, while on all other soils the gain in dry weight due to inoculation averaged 62 percent at the first cutting and only 40 percent at the second cutting. While sterilization doubtless killed all of the soil bacteria present in the soil at the time, accidental inoculation did occur afterwards, as was evidenced by the pres-

ence of root nodules on plants which did not receive the artificial inoculation. This accidental contamination was so small that the bacteria seemingly did not increase nearly as rapidly in the sterilized soil as they did in the same soil not sterilized, where bacteria were present in some quantity before any artificial inoculation was made.

Fig. 3.—Inoculated alfalfa growing in cultivated Rillito River bottom soil.

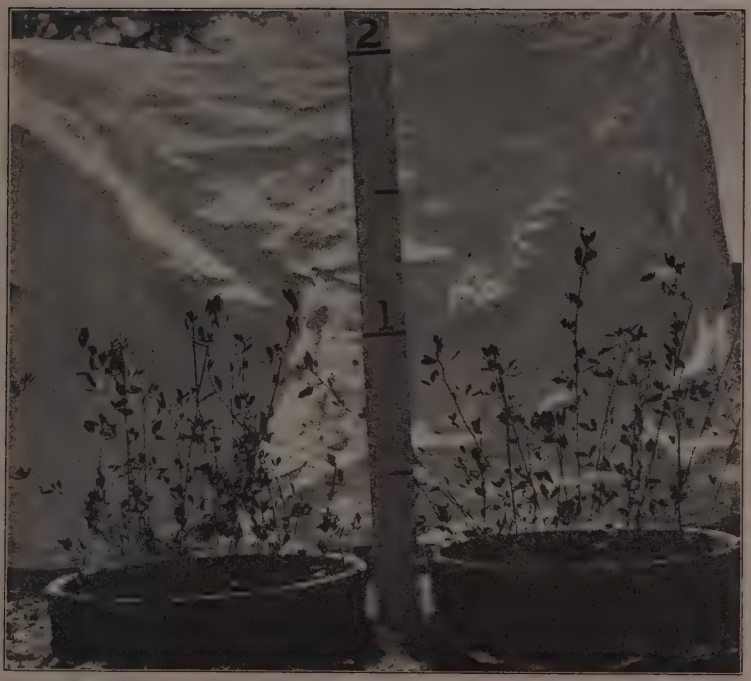

Fig. 4.—Alfalfa plants not inoculated growing in cultivated Rillito River bottom soil.

TABLE V. THE EFFECT OF INOCULATION ON THE DRY WEIGHT AND ON THE NITROGEN CONTENT OF THE TOPS OF ALFALFA PLANTS.

Soil	Treatment	Cutting. No.	Dry Weight		Nitrogen		
			Grams per pot	Percent gain or loss	Percent	Percent gain or loss	Lbs. gain or loss per ton
Virgin Rillito River bottom	inoc.	1	11.50	54.36	2.57		
	not inoc.	1	7.45		2.33	.24	4.8
	inoc.	2	12.00		2.38		
	not inoc.	2	8.75	37.14	2.59	—.21	—4.2
	inoc.	3	1.65				
	not inoc.	3	none				
	inoc.	total	25.15				
	not inoc.	total	16.20	55.24			
Virgin Rillito River bottom steril-ized	inoc.	1	9.25		2.46		
	not inoc.	1	7.05	31.20	1.99	.47	9.4
	inoc.	2	9.10		2.60		
	not inoc.	2	6.00	51.66	2.51	.09	1.8
	inoc.	3	2.4				
	not inoc.	3	none				
	inoc.	total	20.75				
	not inoc.	total	13.05	59.00			
Culti-vated Rillito River bottom	inoc.	1	11.03		2.48		
	not inoc.	1	6.60	67.12	2.38	.10	2.0
	not inoc.	2	8.05		2.35		
	inoc.	2	6.05	33.05	2.40	—.05	—1.0
	inoc.	3	0.9				
	not inoc.	3	none				
	inoc.	total	19.98				
	not inoc.	total	12.65	57.85			
Virgin desert east of campus	inoc.	1	10.75		2.64		
	not inoc.	1	6.48	65.89	2.42	.22	4.4
	inoc.	2	10.20		2.68		
	not inoc.	2	6.80	50.00	2.56	.12	2.4
	inoc.	3	0.9				
	not inoc.	3	none				
	not inoc.	total	21.85				
	inoc.	total	13.28	64.53			

The nitrogen content of the alfalfa tops was slightly greater in every instance in the first cuttings of the inoculated plants than in the corresponding cuttings of the plants not inoculated. This was also true of the second cuttings on the two soils which best responded to inoculation; namely, the desert soil and the sterilized river bottom soil. With both the virgin soil and the cultivated river bottom soils which had not been sterilized, the nitrogen content of the second cutting of plants, which had not been inoculated, was slightly higher than the nitrogen content of those plants which had been inoculated.

TABLE VI. THE EFFECT OF INOCULATION ON THE DRY WEIGHT AND ON THE NITROGEN CONTENT OF THE ROOTS OF ALFALFA PLANTS.

Soil	Treatment	Dry Weight		Nitrogen	
		Grams per pot	Percent gain or loss	Percent	Percent gain or loss
Virgin Rillito River bottom	inoc.	38.25		1.68	
	not inoc.	29.00	31.89	1.92	—.24
Virgin Rillito River bottom sterilized	inoc.	24.50		2.19	
	not inoc.	17.20	42.44	2.03	.16
Cultivated Rillito River bottom	inoc.	48.25		1.61	
	not inoc.	20.40	110.04	2.10	—.49
Virgin desert east of campus	inoc.	30.40		1.68	
	not inoc.	19.95	52.38	2.10	—.42

In every instance the dry weight of the roots of the inoculated alfalfa plants was greater than that of the plants not inoculated, this increase being less with the sterilized soil than with any of the other soils. The nitrogen content of the roots was greater in the plants not inoculated than in those inoculated, with the exception of those grown in sterilized soil, in which instance there was a gain of .16 percent in the inoculated plants over that in the plants not inoculated. This can be explained by the fact that while the third cutting or aftermath was large enough to harvest on the inoculated plants, it was not large enough on the plants not inoculated. The few leaves that did grow on the plants not inoculated were weighed and analyzed with the roots, thus raising materially the percent of nitrogen. There was practically no aftermath on the plants not inoculated which were grown on the sterile soil, and, as a consequence, the nitrogen content in this instance was lower than it was in the roots of the inoculated plants grown on sterile soil.

TABLE VII. THE EFFECT OF INOCULATION OF ALFALFA ON THE NITROGEN CONTENT OF THE SOIL.

Soil	Treatment	NITROGEN					
		Percent at beginning	Percent at close	Percent gain or loss due to inoculation	Percent gain or loss each treatment	Grams gain or loss soil plus roots per pot	Percent gain or loss soil plus roots
Virgin Rillito River bottom	inoc.	.066	.063		—.003	.25	2.92
	not inoc.	.066	.063	none	—.003	.166	1.94
Virgin Rillito River bottom sterilized	inoc.	.066	.063		—.033	.146	1.71
	not inoc.	.066	.064	.001	—.002	.089	1.04
Cultivated Rillito River bottom	inoc.	.066	.068		.002	.949	11.06
	not inoc.	.006	.065	.003	—.001	.298	3.48
Virgin desert east of campus	inoc.	.032	.040		.008	1.55	37.27
	not inoc.	.032	.039	.001	.007	1.33	31.94

The nitrogen content of the cultivated soil of the Rillito River bottom was the same as that of the virgin soil from the Rillito River bottom at the beginning of the experiment, due, probably, to the fact that nearly all of our virgin soils are poor in humic nitrogen. A good rotation system, such as has been used on this cultivated soil, will at least keep the soil from becoming any more deficient in humic nitrogen. It is easily possible to increase the nitrogen content materially, as indicated in the last column of Table VII.

The desert soil contained a trifle less than one-half as much nitrogen at the beginning of the experiment as did the river bottom soils. The desert soil made a considerable gain in nitrogen over what it had in the beginning by growing in it either inoculated or non-inoculated alfalfa plants. This was not true with the other soils, except when inoculated alfalfa was grown in cultivated river bottom soil, in which instance it gained .002 percent. This gain would amount to 40 pounds of nitrogen per acre and does not take into consideration the nitrogen contained in the roots. When the nitrogen in the roots was added to that of the soils at the end of the experiments, all soils showed a gain in nitrogen content, with the desert soil far in the lead. The pots of this latter soil containing the inoculated

plants made a gain of over 37 percent. In each case the gain in nitrogen of the soil and roots combined was greater with the inoculated plants.

TABLE VIII. THE EFFECT OF INOCULATION ON THE DRY WEIGHT AND ON THE NITROGEN CONTENT OF THE TOPS OF SOYBEANS AND COWPEAS.

Crop	Treatment	Dry Weight		Nitrogen		
		Grams per pot	Percent gain or loss	Percent	Percent gain or loss	Lbs. gain or loss per ton
Soybeans cut before formation of seed	inoc.	20.25		1.21		
	not inoc.	9.40	115.4	1.08	.13	2.6
Cowpeas cut before formation of seed	inoc.	38.05		2.74		
	not inoc.	15.45	146.2	1.92	.82	16.4
Cowpeas cut after formation of seed	inoc.	203.40		2.21		
	not inoc.	16.80	1110.0	2.13	.08	1.3

The results obtained with soybeans and cowpeas grown only in the virgin river bottom soil were practically the same as those of the alfalfa grown in the same soil. (See Tables 4, 5, and 6.) The principal difference was that there was a gain of .002 percent of nitrogen in the soil itself with the growing of the cowpeas, while with the alfalfa and the soybeans there was a loss of from .002 to .004, or from 40 to 80 pounds of nitrogen per acre. This loss was more than made up, in the case of the alfalfa, by the addition of the nitrogen contained in the roots, but with the soybeans there was still a loss of nitrogen in the combined roots and soil. The loss in the latter case was .72 percent in the soil growing the inoculated plants and 4.89 percent in the soil growing the plants not inoculated. The roots of the non-inoculated soybean plants had no nodules whatever, while all of the alfalfa and cowpea plants, both inoculated and non-inoculated, had nodules, the nodules being more numerous on the inoculated plants. The total gain in nitrogen when that in the roots was added to that in the soil was much greater with the cowpeas than with the alfalfa, being 8.23 percent with the inoculated cowpeas and 5.98 percent with the cowpeas not inoculated, while the gain was but 2.92 percent and 1.94 percent, respectively, with the alfalfa.

Fig. 5.—The effect of inoculation on the growth of soybeans. The plants in two pots on the right are inoculated, while those on the left are not.

Fig. 6.—The effect of inoculation on the growth of cowpeas. The plants in two pots on the right are inoculated, while those on the left are not.

TABLE IX. THE EFFECT OF INOCULATION ON THE DRY WEIGHT AND ON THE NITROGEN CONTENT OF THE ROOTS OF SOYBEANS AND COWPEAS.

Crop	Treatment	Dry Weight		Nitrogen	
		Grams per pot	Percent gain or loss	Percent	Percent gain or loss
Soybeans cut before formation of seed	inoc.	11.60		1.71.	
	not inoc.	8.75	32.57	1.14	.57
Cowpeas cut before formation of seed	inoc.	29.76		1.50	
	not inoc.	16.88	76.3	1.50	none
Cowpeas cut after formation of seed	inoc.	26.10*		1.41*	
	not inoc.	16.00*	57.22	.92*	.49

In every case the weight of the roots of the alfalfa was greater than the combined weights of the various cuttings of the tops of the alfalfa, averaging 60.5 percent of roots and 39.5 percent of tops. With the cowpeas the weight of the tops averaged 61 percent, while that of the roots averaged 39 percent of the total weight. The root systems of the soybeans averaged 42 percent and the tops 57.7 percent. This difference in the weights of the root systems of various legumes emphasizes the fact that in growing a crop for green manuring purposes the amount of top growth is not always the biggest factor in the matter of improving the soils.

*Roots not washed out of the soil immediately upon harvesting the cowpeas. The roots were partially decayed when finally washed and analyzed. In all other instances the roots were taken from the soil within twenty-four hours after harvesting the tops.

TABLE X. THE EFFECT OF THE INOCULATION OF SOYBEANS AND COWPEAS ON THE NITROGEN CONTENT OF THE SOIL.

Crop	Treatment	NITROGEN						
		Percent at beginning	Percent at close	Percent gain or loss due to inoculation	Percent gain or loss each treatment	Grams gain or loss soil plus roots per pot	Percent gain or loss soil plus roots	Lbs. gain or loss per acre
Soybeans cut before formation of seed	inoc.	.066	.064		—.002	—.062	—.72	—9.5
	not inoc.	.066	.062	.002	—.004	—.42	—4.89	—64.4
Cowpeas cut before formation of seed	inoc.	.066	.068		.002	.71	8.23	108.9
	not inoc.	.066	.068	none	.002	.51	5.98	78.2
Cowpeas cut after formation of seed	inoc.	.066	.068		.002	.63	7.32	96.6
	not inoc.	.066	.068	none	.002	.41	4.81	62.9

TABLE XI. THE EFFECT OF INOCULATION ON THE PERCENT OF ROOTS AND TOPS OF ALFALFA, COWPEAS, AND SOYBEANS. DRY WEIGHT.

Crop	Treatment	PERCENT	
		Tops	Roots
Alfalfa*			
Virgin soil	inoc.	39.6	60.4
Rillito River bottom	not inoc.	35.8	64.2
Virgin soil	inoc.	45.8	54.2
Rillito River bottom	not inoc.	43.1	56.9
Cultivated soil	inoc.	31.6	68.4
Rillito River bottom	not inoc.	38.3	61.7
Virgin soil	inoc.	41.9	58.1
desert east of campus	not inoc.	39.9	60.1
Cowpeas**			
Cut before formation of seed	inoc.	56.3	43.7
	not inoc.	47.8	52.2
Cut after formation of seed	inoc.	89.7	11.3
	not inoc.	50.3	49.7
Soybeans**			
Cut before formation of seed	inoc.	63.6	36.4
	not inoc.	51.8	48.2

*All cuttings of the alfalfa tops are included. In the case of the cowpeas and soybeans but one cutting was made.

**Virgin Rillito River bottom soil unsterilized was the only soil used with the cowpeas and soybeans.

TABLE XII. THE AMOUNT OF NITROGEN TAKEN FROM THE SOIL AND THAT TAKEN FROM THE AIR FOR THE USE OF THE PLANTS.

Crop	Treatment	Grams in tops	Grams in roots	Total in plants	Grams in soil at beginning	Grams in soil at end	Loss or gain in soil	Grams taken from soil by plants	Grams taken from air by plants	Percent taken from air
Alfalfa										
Virgin soil—Rillito River bottom	inoc.	.62	.64	1.26	8.58	8.19	—.39	.39	.87	69
	not inoc.	.40	.56	.96	8.58	8.19	—.39	.39	.37	58
Virgin soil—Rillito River bottom sterilized	inoc.	.53	.54	1.07	8.58	8.19	—.39	.39	.38	54
	not inoc.	.35	.35	.70	8.58	8.32	—.26	.26	.44	63
Cultivated Rillito River bottom soil	inoc.	.48	.69	1.17	8.58	8.84	.26	none	1.43	125
	not inoc.	.30	.43	.73	8.58	8.45	—.13	.13	.60	82
Virgin desert soil	inoc.	.58	.51	1.09	4.16	5.2	1.04	none	2.13	195
	not inoc.	.33	.42	.75	4.16	5.07	.91	none	1.66	221
Cowpeas										
Cut before formation of seed	inoc.	1.04	.45	1.49	8.58	8.84	.26	none	1.75	117
	not inoc.	.30	.25	.55	8.58	8.84	.26	none	.81	147
Cut after formation of seed	inoc.	4.50	.37	4.87	8.58	8.84	.26	none	5.13	105
	not inoc.	.36	.15	.51	8.58	8.84	.26	none	.77	151
Soybeans										
Cut before formation of seed	inoc.	.25	.20	.45	8.58	8.32	—.26	.26	.19	42
	not inoc.	.10	.10	.20	8.58	8.06	—.52	.52	.00	0

The cowpeas obtained a greater percent of nitrogen from the air than did either the alfalfa or the soybeans. In the case of the soybeans more nitrogen was obtained from the soil than from the air, the inoculated plants having obtained 42 percent from the air and the remainder from the soil. With the cultivated river bottom soil, the inoculated plants of alfalfa obtained more than enough nitrogen for their own use from the air; this was also true for both the inoculated plants and those not inoculated that were grown on desert soil. With the alfalfa the inoculated plants in all cases, except when grown on desert soil, obtained a larger percent of their nitrogen from the air than did the plants not inoculated.

TABLE XIII. THE EFFECT OF APPLYING INOCULATION MATERIAL AT DIFFERENT RATES ON THE WEIGHT IN GRAMS OF THE TOPS AND ROOTS OF ALFALFA.

Treatment	Tops	Roots	Total
Soil not sterilized			
seed not inoculated	.722	.318	1.040
*Soil sterilized			
seed not inoculated	.388	.614	1.002
seed given normal inoculation	.255	.201	.456
seed given 10 times normal inoculation	.433	.393	.826
seed given 20 times normal inoculation	.791	.700	1.491

Twelve pots containing uniform virgin Rillito River bottom soil were planted to alfalfa, the seed of which had been treated with different amounts of the inoculation material to ascertain the effect of an excess application. Nitrosoil inoculum was used for this test, but it was not as fresh as it should have been. The nodules on the roots of the alfalfa grown in the soil not sterilized were much larger as well as more numerous than those on the roots of the plants given any of the other treatments, except that in the case of the pots receiving twenty times the normal application of inoculation material, the nodules were as numerous, but were very small. The nodules resulting from action of the bacteria already in the soil were many times larger in every instance than were those resulting from action of the bacteria applied in the artificial inoculum. This has been noted in practically all of the inoculation experiments conducted by the Experiment Station during the last few years.

A study of Table 13 shows that inoculation did not increase the weight of the tops or the total plant weights with the normal application or with ten times the normal application of inoculation material, but did increase the weights with an application of twenty times the normal. These results empha-

*Sterilized in autoclave for one hour at 20 pounds pressure.

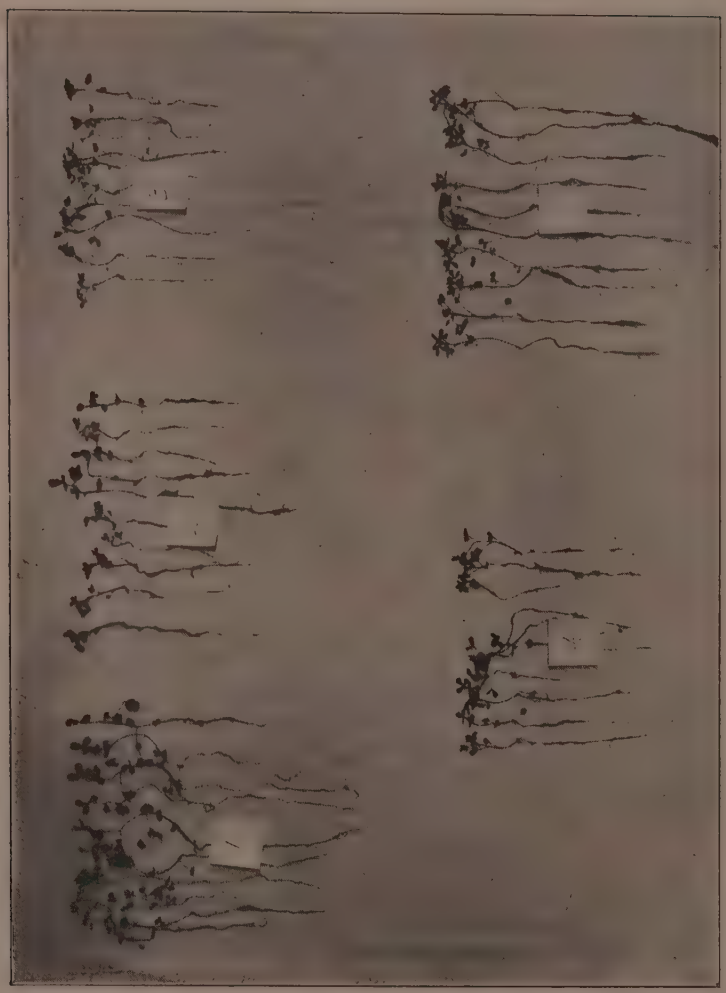

Fig. 7.—The effect of applying inoculation material at different rates. (1) Soil not sterilized, plants not inoculated; (2) soil sterilized, plants not inoculated; (3) soil sterilized, plants given normal inoculation; (4) soil sterilized, plants given 10 times normal inoculation; (5) soil sterilized, plants given 20 times normal inoculation.

size the necessity, when making pot culture tests, of using the amount of inoculation material recommended by the companies which put out this material. The tendency is for one to use as much inoculum on a few pot cultures as is recommended by the manufacturing company for perhaps an acre, and to judge the results in the light of normal applications.

Fig. 8.—The difference between natural and artificial inoculation on the formation of root nodules. The nodules on the roots of the two plants on the left were formed through the action of bacteria already present in the soil; the nodules on the roots of the two plants at the right were formed by bacteria introduced in commercial inoculum and are too small to be seen in a photograph, in most cases being smaller than a pinhead.

SUMMARY

Inoculation has not proved beneficial with alfalfa at the Salt River Valley Experiment Farm.

In most instances inoculation has been beneficial at the Sulphur Spring Valley Dry-Farm.

Increase in yield of vetch due to inoculation has been marked on the very sandy soil of the Yuma Mesa Farm. This increase was greater during the first year of cultivation than during the second. In soil where inoculated vetch had been grown the preceding year, the inoculation of vetch seed increased the yields very materially over vetch seed not inoculated.

The weight of nodules on the roots of the vetch plants which had not been inoculated was greater than on those which had been inoculated, while the number of nodules per plant was less.

Inoculation hastened maturity from five to seven days with alfalfa grown in pots at the University campus in the screen gardens.

The weights of the tops and of the roots of all legumes experimented with in the pot tests were increased materially by inoculation.

Inoculation increased the weight of nitrogen in the tops of the alfalfa from one to nine pounds per ton, depending on the soil used. The gain in nitrogen content of the cowpea hay was 16 pounds per ton, and but 2.6 pounds per ton with soybeans.

In poor desert soil or in Rillito River bottom soil which had been in cultivation for a number of years a distinct gain in the nitrogen content of the soil itself was obtained by growing alfalfa, either inoculated or not inoculated. With the other two soils there was a loss in nitrogen content.

When the nitrogen content of the roots was added to that of the soil, in every instance with the alfalfa there was a gain of from 1.04 percent in the case of the virgin Rillito River bottom soil to 37.27 percent with the desert soil. Cowpeas grown on the virgin soil of the Rillito River bottom gained from 6 to 8 percent nitrogen, while soybeans incurred a loss of .72 to 4.89 percent.

Inoculation increased the percent of roots to tops with alfalfa, but had the opposite effect with cowpeas and soybeans.

Nodules produced on the roots of vetch and alfalfa plants by the action of bacteria already present in the soil, are as a rule larger than those produced by the bacteria in commercial cultures.

Root nodules cannot be found in nearly so great abundance during the hot summer months as during cooler weather.

An application twenty times normal of commercial inoculum in sterile soil gave a yield of alfalfa three times as heavy as a normal application.

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